Attorney Docket No. 5405.225

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Boustany et al.
Serial No.: 09/830,045
Filed: January 23, 2002
For: *Methods of screening for risk of proliferative disease and methods for the treatment of proliferative disease*

Group Art Unit: 1634
Examiner: J. Goldberg

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Rose-Mary N. Boustany, M.D. Pursuant to 37 C.F.R. § 1.132

I, Rose-Mary N. Boustany, do hereby declare and say as follows:

1. I am a named inventor on United States Application No. 09/830,045 ("the '045 application") and of the subject matter claimed therein.

2. I have a Medical Degree (M.D.) from the American University of Beirut and received my subspecialty training at the Mass General in Boston at Harvard Medical School. I am a Professor of Pediatrics and Neurobiology at Duke University in Durham, North Carolina, and a Professor of Pediatrics and Biochemistry at the American University of Beirut. I have been conducting research in the area of Batten's disease, familial spastic paraplegia, Tay-Sachs disease, GM-1, gangliosidosis and other lysosomal storage diseases, as well as the role of the CLN3 gene in these and other disorders such as proliferative disorders for 19 years and have co-authored more than 65 publications related to this area.

3. The studies described below were carried out in my laboratory at Duke University in Durham, North Carolina, under my direction and supervision according to the disclosure set forth in the '045 application. These studies demonstrate a correlation between upregulation of the CLN3 gene in humans and colon cancer.

4. The following experimental studies were performed. Samples of human colon cancer and normal colon tissue from the same patient were obtained using an Institutional Review Board-approved procedure for collecting leftover, "deidentified," or "unlinked" specimens from surgical pathology. In brief, at the time of frozen section analysis of the patient tumor, samples of tumor and normal colon tissue not needed for diagnostic purposes were snap frozen in embedding medium and stored at -80°C. The

samples were deidentified by removing all of the patient information from the samples except for a pathological diagnosis. Frozen sections of each specimen were stained with hemotoxylin and eosin and examined by a pathologist to insure that the frozen samples contained representative normal or neoplastic tissue. In all of the cases, the colon cancer specimens consisted mostly of neoplastic glands invading into the muscularis externa. The normal colon specimens contained mucosa, submucosa, and muscularis externa.

CLN3 mRNA expression was measured in human colon tumors and compared with CLN3 mRNA expression in normal colon tissue obtained from the same patient at the time of surgery. CLN3 mRNA was isolated from 10 solid colon cancers, and expression of CLN3 was compared with normal colon tissue obtained from the same patient using RT-PCR. Cyclophilin was used as an internal control. Results were expressed as percentage change of CLN3 expression in colon cancer samples compared with corresponding normal colon controls. Each experiment was repeated twice.

Data from these experiments demonstrated that in seven of the ten tumors, CLN3 expression was 50–330% higher than in the corresponding normal colon tissue and in an eighth case, CLN3 expression was increased by 22%. Overexpression of CLN3 in 80% of solid colon cancers tested corroborates data obtained from human colon cancer cell lines as described in the '045 application.

These data demonstrate a correlation between increased CLN3 expression in humans and colon cancer and thus provide a method for identifying human subjects at increased risk of developing colon cancer on the basis of upregulation of the CLN3 gene in such subjects.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Rose-Mary N. Boustany, M.D.

3/27/2006
Date